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Three new species of lichenized fungi from Qinghai Province, China

Beeyoung Gun Lee[1], Sergij Y. Kondratyuk[2], Josef P. Halda[3], László Lőkös[4], Hai-Ying Wang[5], Min Hye Jeong[1], Sangkuk Han[6], Soon-Ok Oh[6], Jae-Seoun Hur[1*]

[1] *Korean Lichen Research Institute, Sunchon National University, Suncheon 57922, Korea*
[2] *M. H. Kholodny Institute of Botany, Tereshchenkivska str. 2, 01004 Kiev, Ukraine*
[3] *Muzeum a galerie Orlických hor, Jiráskova 2, 516 01 Rychnov nad Kněžnou, Czech Republic*
[4] *Department of Botany, Hungarian Natural History Museum, H-1431, Budapest, Pf. 137, Hungary*
[5] *College of Life Sciences, Shandong Normal University, Jinan 250014, China*
[6] *Korean National Arboretum, 415 Gwangneungsoomokwon-ro, Soheul-eup, Pocheon 11186, Korea*

* Correspondence to: *jshur1@sunchon.ac.kr*

Abstract—Three new species of lichenized fungi—*Calogaya qinghaiensis, Caloplaca zeorina*,*Verrucaria eminens*—are described from Qinghai province, China, and compared with closely related species.

Key words—biodiversity, *Teloschistaceae*, *Verrucariaceae,* taxonomy

Introduction

Qinghai province is located at 31–39°N 89–103°E on the northeast Tibetan Plateau. The province consists of mountains and high plateaus with an average 3000 m elevation (the lowest basin approximately 2700 m a.s.l.) and featuring numerous lakes, including Qinghai Lake, the second largest saltwater lake in the world (Encyclopedia Britannica 2015, China.org.cn 2015). Such a variable environment, encompassing both xerophytic and halophytic conditions, supports a rich lichen diversity that remains less well explored than in the surrounding provinces of Gansu, Sichuan, Xizang, and Xinjiang.

The earliest explorations and surveys of the lichenological flora of Qinghai were conducted by G.N. Potanin in 1876–94 and B. Bohlin in 1930–32 (Wei 1991), and new species and records continue to be reported from this region in the 21st century (Fu & al. 2009; Hou & al. 2014; Liu & al. 2016; Wang & al. 2012, 2015).

During a 2014 summer field excursion to Qinghai Province organized by Dr. H.-Y. Wang of Shandong Normal University, three specimens were collected that were identified to genus but did not correspond with any of the described species. We propose them here as new species: *Calogaya qinghaiensis*, *Caloplaca zeorina*, and *Verrucaria eminens*. All specimens collected on the 2014 excursion were first deposited in the herbarium of the Korean Lichen Research Institute, Suncheon, Korea (KoLRI) and then transferred to the herbarium of the Korea National Arboretum (KH), Korea, in 2016.

Materials & methods

The specimens were examined morphologically under a Nikon SMZ645 stereo-zoom dissecting microscope, and thin hand-cut sections were made with a razor blade for observation of anatomical features under a Nikon Eclipse E200 compound microscope. Anatomical mages were captured with the AxioVison Release 4.8.2 software program and Axiocam ERc 5s camera mounted on a Zeiss A1 microscope. Length and diameter of at least ten ascospores per specimen were measured in water at 400× magnification. Thin-layer chromatography (TLC) was performed in Solvent systems A and C as per Orange & al. (2001), and HPLC was conducted as per Søchting (1997).

DNA was extracted from all specimens representing new and closely related species employing the DNA extraction kit NucleoSpin® Plant II. The full internal transcribed spacer (ITS) region (ITS1+5.8S +ITS2 rDNA) was amplified using Bioneer's AccuPower PCR Premix in 20 µl tubes employing primers ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990). PCR thermal cycling parameters followed Ekman (2001). Additionally, DNA was cloned using the pGEM®-T Easy Vector Systems when PCR and electrophoresis results were unclear or double bands were observed. The PCR products were sequenced by GenoTech (Daejeon, Korea), and the sequences were aligned and manually edited using ClustalW in Bioedit (V7.2.5).

For phylogenetic analysis, all DNA sequences were arranged and edited in MEGA6. Bootstrap values were obtained in ClustalX 2.1 (Larkin & al. 2007) and RAxML GUI 1.5 beta (Silvestro & Michalak 2010) using Maximum Likelihood (ML) with a rapid bootstrap, 1000 bootstrap replications, and GTR GAMMA for the substitution matrix. Posterior probabilities were produced in BEAUti 1.8.0 and BEAST 1.8.0 (Drummond & al. 2012) employing the HKY method for the substitution model, empirical base frequency, gamma for site heterogeneity model and four categories

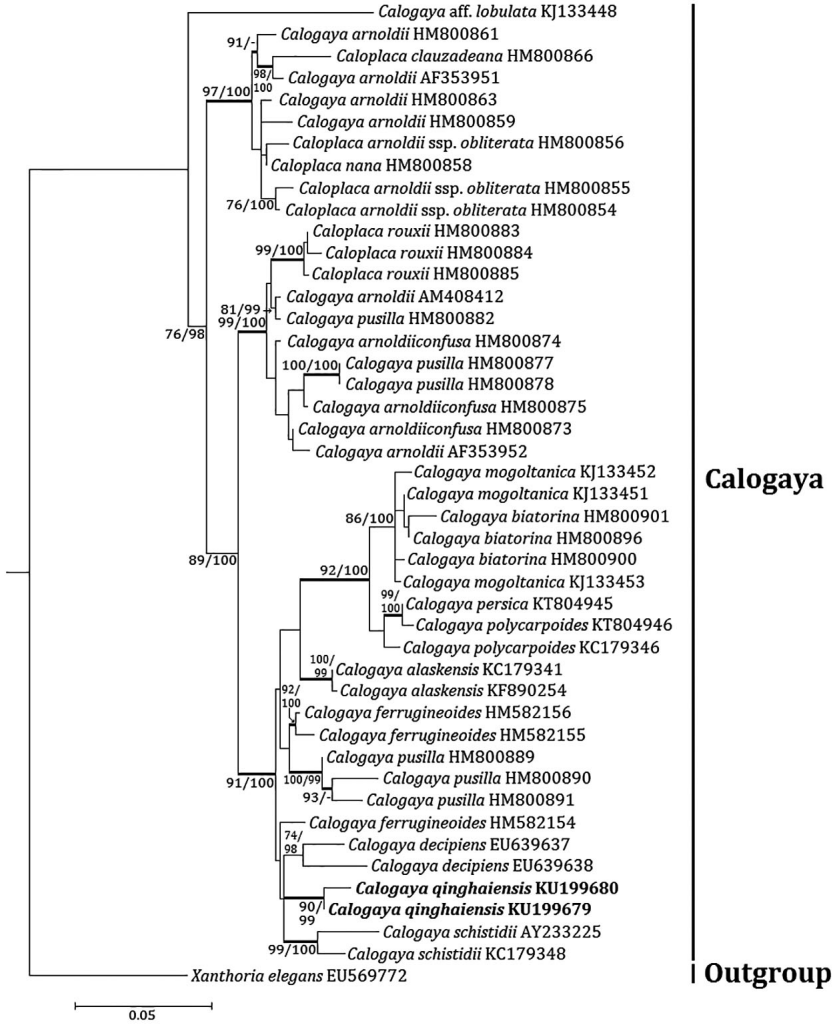

Plate 1. Phylogenetic relationships in *Calogaya*, based on a Maximum Likelihood analysis of the nuclear ribosomal ITS1-5.8S-ITS2 region. The tree was rooted using *Xanthoria elegans*. Maximum Likelihood bootstrap values ≥70% and posterior probabilities ≥95% are shown above internal branches. Branches with bootstrap values ≥90% are in bold. New sequences are presented in bold and all species names are followed by GenBank accession numbers. A dash indicates branches with posterior probabilities <95%.

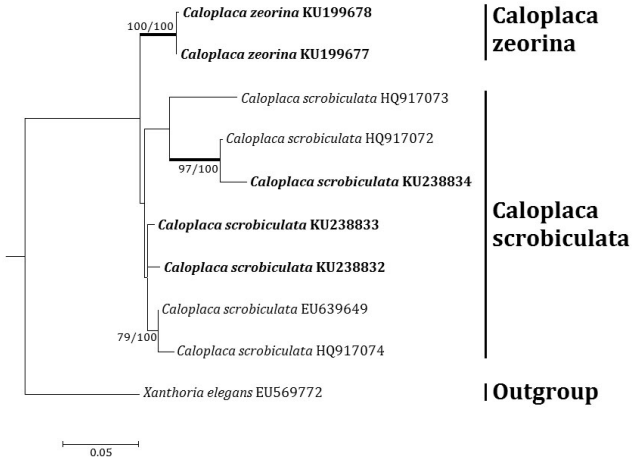

Plate 2. Phylogenetic relationships among compatible species surrounding *Caloplaca scrobiculata*, based on a Maximum Likelihood analysis of the nuclear ribosomal ITS1-5.8S-ITS2 region. The tree was rooted using *Xanthoria elegans*. Maximum Likelihood bootstrap values ≥70% and posterior probabilities ≥95% are shown above internal branches. Branches with bootstrap values ≥90% are in bold. New sequences are presented in bold and all species names are followed by GenBank accession numbers. A dash indicates branches with posterior probabilities <95%.

for gamma, and one million MCMC (Markov Chain Monte Carlo) chain length with ten thousand echo state screening and two hundred log parameters. The best tree was determined using TreeAnnotator 1.8.0 (Drummond & Rambaut 2007) with a burn-in of one hundred, no posterior probability limit, a maximum clade credibility tree for target tree type, and median node heights. All trees were illustrated in FigTree 1.4.2 (Rambaut 2014) and edited in Microsoft Paint.

Results & discussion

The *Calogaya* phylogenetic tree (Pl. 1) was produced from 43 GenBank sequences (34 for *Calogaya*, eight for *Caloplaca*, one for an outgroup *Xanthoria elegans*) and two new sequences from our new species, *C. qinghaiensis*. *Calogaya qinghaiensis* is positioned within *Calogaya* (previously *Caloplaca saxicola* group), which comprises *C. alaskensis* (Wetmore) Arup & al., *C. arnoldii* (Wedd.) Arup & al. (including several subspecies), *C. arnoldiiconfusa* (Gaya & Nav.-Ros.) Arup & al., *C. biatorina* (A. Massal.) Arup & al., *C. decipiens* (Arnold) Arup & al., *C. ferrugineoides* (H. Magn.) Arup & al., *C. lobulata* (Flörke) Arup & al., *C. mogoltanica* (S.Y. Kondr. & Kudratov) S.Y. Kondr. & al., *C. persica* (J. Steiner) Arup & al., *C. polycarpoides* (J. Steiner) Arup & al., *C. pusilla* (A. Massal.) Arup

& al., *C. schistidii* (Anzi) Arup & al., and *Caloplaca rouxii* Gaya & al. *Calogaya qinghaiensis* forms a well supported monophyletic clade, but our analysis did not reveal any clear relationship with adjacent clades.

The phylogenetic tree for the *Caloplaca scrobiculata* group (Pl. 2) was produced from five GenBank sequences (four for *Caloplaca* spp., one for an outgroup *X. elegans*) and five new sequences (two for *C. zeorina,* three for *C. scrobiculata*). Our analysis supports *Caloplaca zeorina* as a sister clade to *C. scrobiculata*.

A phylogenetic tree for *Verrucaria* (not shown), including our new sequences from *V. eminens* and *V. pinguicula*, placed *V. eminens* as basal to all other sequenced *Verrucaria* species but with very low support.

Taxonomy

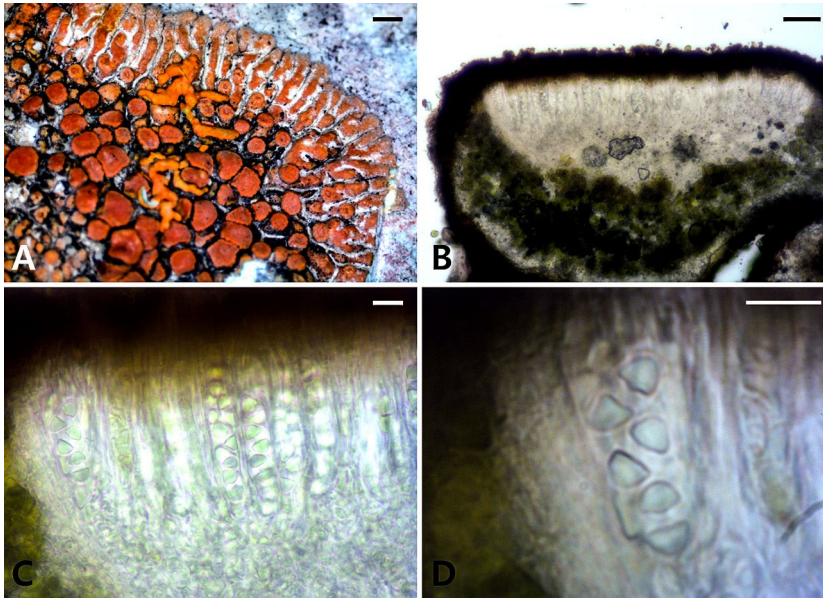

Plate 3. *Calogaya qinghaiensis* (holotype, KoLRI 024154). A. habitus; B. section through apothecium; C, D. asci and ascospores. Scale bars: A = 1 mm; B = 50 µm; C, D = 10 µm.

Calogaya qinghaiensis B.G. Lee & Hur, **sp. nov.** Pl. 3

MycoBank MB 815074

Differs from *C. decipiens* by an absence of soralia/soredia, its frequent apothecia, and its reddish apothecial discs.

Type: China, Qinghai province, Tianjun County, Yangkang village, 37°43′15″N 98°32′09″E, 3688 m, 28 July 2014, S.O. Oh, S.K. Han, & J.S. Hur CH140258 (**Holotype**, KoLRI 024154; GenBank KU199679).

Etymology: The species epithet indicates the collection locality, Qinghai.

Morphology—Thallus crustose, reddish-orange, 1.7–2.4 cm diam., pruinose, placodioid, margin abrupt at edge. Lobes convex, marginally narrow, 1.7–2.5 × 0.4–0.8 mm, surface white pruinose. Cortex cellular, 25–32.5 µm thick, with granules insoluble in K. Medulla prosoplectenchymatous, 90–100 µm high, with granules insoluble in K. Apothecia adnate, 0.3–0.9 mm diam., lecanorine. Disc reddish-orange, flat, mainly epruinose or slightly white pruinose. Thalline margin persistent, slightly raised above disc level. Proper margin thin, concolorous with disc. Epihymenium brown to light brown, 30.0–33.8 µm tall, K+ purple. Hymenium hyaline, 49.1–75.0 µm tall. Subhymenium hyaline, 28.8–47.1 µm tall. Hypothecium hyaline, 44.3–54.3 µm tall. Paraphyses simple, unbranched, with 1–2 swollen tip cells. Asci cylindrical, 8-spored. Ascospores hyaline, ellipsoid, 10–12.5 × 5–6.25 µm, septum 1.5–3 µm high, spore end wall thin. Pycnidia not observed.

Chemistry—Thallus and apothecial disc K+ purple, C–, KC–, P–. UV+ red to dark orange. Parietin detected by TLC and HPLC.

Ecology & distribution—So far known only from its type locality where it grows on rocks associated with *Caloplaca scrobiculata, Oxnerella safavidiorum* S.Y. Kondr. & al., and *Xanthoria elegans* (Link) Th. Fr.

Additional specimen examined: **CHINA**, **Qinghai province**, **Tianjun County,** Yangkang village, 37°43′15″N 98°32′09″E, 3688 m, 28 July 2014, S.O. Oh, S.K. Han, & J.S. Hur CH140262 (KoLRI 024158; GenBank KU199680).

Remarks—*Calogaya qinghaiensis* shares morphological similarities with *C. arnoldii*, *C. biatorina*, *C. decipiens*, *C. ferrugineoides*, *C. pusilla,* and *C. schistidii*.

Calogaya arnoldii differs from *C. qinghaiensis* by its smaller thallus, irregularly arranged marginal lobes, no differentiation of both proper and thalline margins, thinner epihymenium, and wider ascospore septa (Gaya 2009); *C. biatorina* is distinguished by its epruinose thallus, mostly flat lobes, and larger ascospores (Gaya 2009); and *C. decipiens* differs in its dark orange apothecia, presence of soralia/soredia, larger ascospores, and the presence of emodin (Gaya 2009).

Calogaya ferrugineoides differs from *C. qinghaiensis* by its colonization of bark, cork, trunks, branches, or twigs, its grayish colored thallus with inconspicuous thallus margin, and the absence of parietin (LIAS light 2018);

C. pusilla is distinguished by unbranched paraphysis tips, slightly larger ascospores, wider septa, and the presence of fallacinal, emodin, teloschistin and parietin (Nash III & al. 2007); and *C. schistidii* differs in its bryophytic substrates (mosses and liverworts), inconspicuous thallus margin, epruinose thallus, yellow epihymenium, and larger ascospores (Gaya 2009).

Caloplaca zeorina B.G. Lee & Hur, **sp. nov.** Pl. 4

MycoBank MB 815073

Differs from *C. anularis* by the presence of zeorin, its completely divided biloculate spores, and living central areoles.

Type: China, Qinghai prov., Haidong prefecture, Mt. Dabanshan, 37°21′04″N 101°24′25″E, 3792 m, 25 July 2014, S.O. Oh, S.K. Han, & J.S. Hur CH140080 (**Holotype**, KoLRI 023976; GenBank KU199677, KU199678).

Etymology: The species epithet indicates the chemical characteristic of zeorin containment.

Morphology—Thallus crustose, reddish-orange, 0.5–0.75 cm diam., up to 480 µm thick, placodioid, margin abrupt at edge. Lobes convex, marginally narrow, 0.7–2.2 × 0.2–0.8(–1.1) mm, surface smooth, white epruinose. Cortex amorphous, 62.5–100 µm thick, with granules insoluble in K. Medulla prosoplectenchymatous, dense, 70–100 µm high, with granules insoluble in K. Apothecia adnate, 0.4–1.1 mm diam., lecanorine. Disc red to orange. Proper margin visible, darker than the disc. Epihymenium brown to light orangish brown, 20.5–38.6 µm tall, K+ purple. Hymenium hyaline, 53.0–76.6 µm tall. Subhymenium hyaline, 60.0–74.6 µm tall. Hypothecium hyaline, 56–100 µm tall. Paraphyses simple, unbranched, with 1–2 swollen tip cells. Asci cylindrical, 8-spored. Ascospores hyaline, ellipsoid, 14–16 × 5.5–10 µm, completely separated into two locules by a narrow septum, ≤1 µm long. Pycnidia not observed.

Chemistry—Thallus and epihymenium K+ red-purple, C–, KC–, P– by spot test, UV+ red to dark orange; parietin, teloschistin, and zeorin detected by TLC; parietin detected by HPLC (teloshistin and zeorin not in the HPLC library).

Ecology & distribution—*Caloplaca zeorina* occurs on rock and is currently known only from the type collection (Qinghai province, China).

Additional specimens examined: ***Caloplaca scrobiculata***: **China**, **Qinghai province**, **Tianjun County,** Yangkang village, 37°43′15″N 98°32′09″E, 3688 m, 28 July 2014, S.O. Oh, S.K. Han, & J.S. Hur CH140280 (KoLRI 024176; GenBank KU238832), CH140273 (KoLRI 024169; GenBank KU238833), CH140283 (KoLRI 024179; GenBank KU238834).

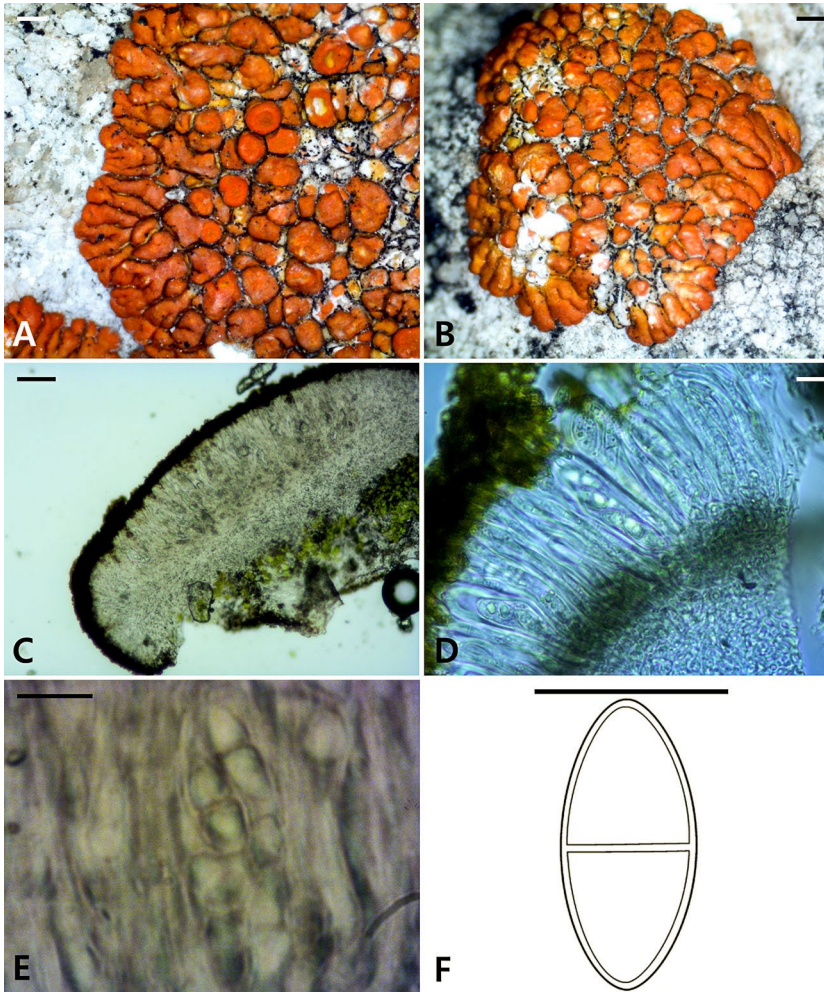

Plate 4. *Caloplaca zeorina* (holotype, KoLRI 023976). A, B. habitus; C, D. section through apothecium; E. asci and ascospores; F. ascospore. Scale bars: A, B = 1 mm; C = 50 μm; D–F = 10 μm.

Remarks—*Caloplaca zeorina* contains the triterpenoid zeorin, the presence of which separates it from other *Caloplaca* species, which typically contain anthraquinones such as parietin, fallacinal, emodin, and/or teloschistin.

Caloplaca zeorina shares several similarities with *C. scrobiculata* and *C. anularis* Clauzade & Poelt. The synonymy of *Caloplca anularis* with

C. scrobiculata by Poelt & Hinteregger (1993) was accepted by a number of authors and cited in the checklists for Austria (Hafellner & Türk 2001), Bulgaria (Mayrhofer & al. 2005), France (Roux 2012), Italy (Nimis & Martellos 2008), Montenegro (Knežević & Mayrhofer 2009), Slovakia (Pišút & al. 1996), and Slovenia (Suppan & al. 2000). However, Khodosovtsev & al. (2004) and Vondrák & Mayrhofer (2013) argued convincingly that these two *Caloplaca* species are quite different from each other and should be treated separately.

Caloplaca zeorina shares more similarities with *C. anularis* than with *C. scrobiculata*: *C. anularis* differs in its yellow to slightly orange-yellow colored and thicker thallus, thicker medulla, narrower ascospores with an undivided single locule and constricted in the middle, and its central areoles that die and fall off, leaving only the margins and displaying ring-shaped thallus fragments (Vondrák & Mayrhofer 2013).

Although possessing ascospores that are quite similar to those found in *C. zeorina*, *C. scrobiculata* is distinguished by its yellow to orange-yellow colored pruinose thallus, absence of a cortical layer and a marginal lobe, longer and narrower ascospores, and the absence of zeorin (Vondrák & Mayrhofer 2013).

Caloplaca zeorina can be confused with *C. ignea* Arup (≡ *Polycauliona ignea* (Arup) Arup & al.) and *C. rouxii,* which also inhabit a rock substratum and produce a thallus that is thick with convex marginal lobes, smooth, reddish, epruinose, and gives a K+ purple reaction. *Caloplaca ignea* is distinguished by its flatter, longer lobes, much thinner cortex, smaller ascospores with a wider septum, the presence of pycnidia, and the absence of zeorin (Nash III & al. 2007). *Caloplaca rouxii* differs in producing a thallus loosely attached to the substratum, shorter and narrower lobes, discolored patches on upper surface, a thinner thallus, cortex, medulla, and algal layer, an apothecial margin color lighter than the disc, a much thinner epihymenium, narrower ascospores with a wider septum, and the absence of zeorin (Gaya 2009).

Verrucaria eminens B.G. Lee & Hur, **sp. nov.** Pl. 5

MycoBank MB 815075

Differs from *V. pinguicula* by a rimose thallus with blackish brown or green pigments, distinctive ostiolar region, and small, round ascospores.

Type: China, Qinghai province, Haidong prefecture, Mt. Dabanshan, 37°20′17″N 101°23′53″E, 3745 m, 25 July 2014, S.O. Oh, S.K. Han, & J.S. Hur, CH140035 (**Holotype**, KoLRI 023931; Genbank KU199681).

Etymology: The species epithet refers to the prominent, large perithecia.

Morphology—Thallus, 272–352 µm thick, rimose, wrinkled, with numerous cracks, dull-white colored, cracks with blackish brown or green pigments (perhaps an algal layer shown inside the cracks), epinecral layer present, pale brown, 7.9–8.7 µm tall. Cortex hyaline or slightly darkened, 11–13.6 µm tall. Algal layer continuous to irregularly discontinuous or isolated in medulla, 80.4–86.6 µm tall. Photobiont cells 7.5 × 5 µm thick. Medulla 38.9–47.6 µm tall. Perithecia prominent (118.3–182.5 µm), sometimes immersed (97.9–113.8 µm), (243–)318(–419) µm diam., often in groups of 2–3, rarely solitary, concave with rounded apex, black. Ostiolar region whitish to pale brown, protruding as a low papilla, c. 200 µm diam. Involucrellum well developed, partly lateral, black, reaching up to the base of the exciple. Periphyses irregularly septate, diameters approximately 29 µm at the base and 42.5–55 µm at the ostiolar region. Asci 30–45 × 17.5–20 µm, 8-spored. Ascospores ellipsoidal to globose, 10.4–15.0 × 8.0–11.1 µm (mean = 12.3 × 9.6; n = 26).

Chemistry—K–, C–, KC–, P–. UV–. No substances detected by TLC.

Ecology & distribution—The species occurs with *Caloplaca* species on calcareous rocks in sunny areas. This species is currently known only from the type collection.

Additional specimen examined: ***Verrucaria pinguicula*** A. Massal.: **SOUTH KOREA**, **Gangwon province**, **Jeongseon-gun,** Jeongseon-eup, Aesan-ri, 37°22′17″N 128°40′25″E, 320 m, 06 Sep 2015, B.G. Lee, J.J. Woo, & J.S. Park 152773 (KoLRI 037760; Genbank KU215797).

Remarks—*Verrucaria eminens* is not easily confused with *V. caerulea* DC., *V. canella* Nyl., *V. dufourii* DC., *V. muralis* Ach., and *V. pinguicula* despite sharing common features such as their substrate preference for calcareous rocks, ±superficial visibly cracked, indistinct prothallus and ±prominent perithecia. However, *V. caerulea* is separated by its non-rimose, pale gray to dull gray-brown, and thinner thallus, less prominent, smaller perithecia, and larger and ellipsoid ascospores (Smith & al. 2009); *V. canella* differs in its thicker pruinose thallus with discrete areoles, three-quarters to completely immersed perithecia, absence of an involucrellum, larger and oblong to ellipsoid ascospores, and the presence of a perispore (Smith & al. 2009); *V. dufourii* is distinguished by its immersed thallus, an inconspicuous ostiolar region, an involucrellum that does not reach the exciple base, larger and oblong to ellipsoid ascospores, and the presence of pycnidia (Smith & al. 2009); and *V. muralis* differs in its thallus usually without pigments, an involucrellum that does not usually reach the exciple base, and larger and oblong to ellipsoid ascospores (Smith & al. 2009).

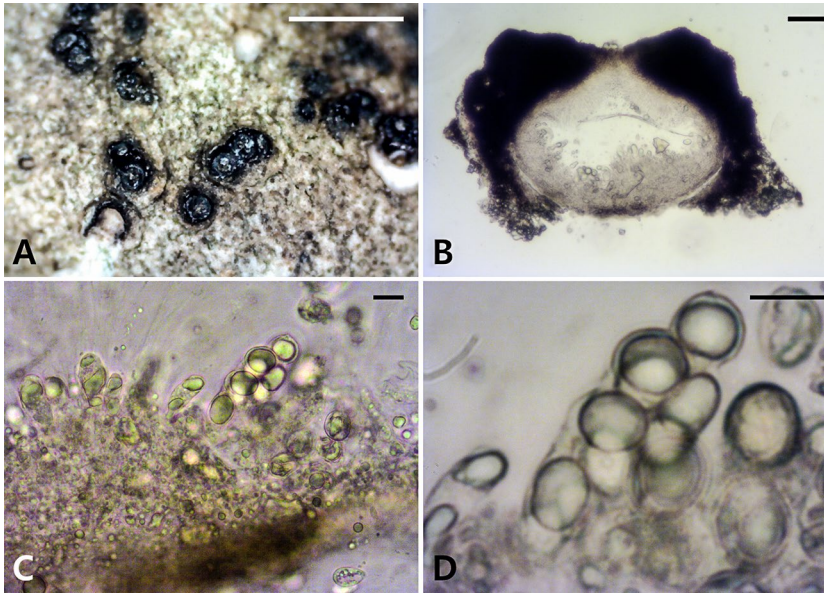

Plate 5. *Verrucaria eminens* (holotype, KoLRI 023931). A. habitus; B. section through perithecium; C. ascoma position; D. ascospores in an ascus. Scale bars: A = 1 mm; B = 50 µm; C, D = 10 µm.

Verrucaria pinguicula, which also produces prominent large perithecia, differs in its superficial or almost immersed thallus, inconspicuous ostioles, and larger (13.5–18 × 5.5–8.5 µm) oblong-ellipsoid spores (Smith & al. 2009).

Acknowledgments

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